Research in Veterinary Science 93 (2012) 807–810

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Occurrence of *Giardia duodenalis* infection in chinchillas (*Chincilla lanigera*) from Italian breeding facilities

F. Veronesi [a,*], D. Piergili Fioretti [a], G. Morganti [a], A. Bietta [b], I. Moretta [a], A. Moretti [a], D. Traversa [c]

[a] Department of Biopathological Sciences and Hygiene of Animal and Food Productions, Section of Parasitology, Faculty of Veterinary Medicine, University of Perugia, Italy
[b] Department of Veterinary Pathology, Diagnostic and Clinics, Section of Experimental Science and Applied Biotechnologies, Faculty of Veterinary Medicine, University of Perugia, Italy
[c] Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, University of Teramo, Italy

ARTICLE INFO

Article history:
Received 11 November 2011
Accepted 23 December 2011

Keywords:
Chinchilla
Giardia
Assemblages
Italy
Breeding facilities
Zoonosis

ABSTRACT

The present work investigated the occurrence of *Giardia* infection in *Chinchilla lanigera* reared in three Italian breeding facilities and determined their role as potential zoonotic reservoir. One hundred and four fecal samples were tested for the presence of *Giardia* spp. cysts using a Direct Fluorescent Assay (DFA). A high positivity rate (39.4%) was found despite all animals were asymptomatic at the time of sampling. Thirty-one positive samples were genetically characterized by sequence analysis of the ITS1–5.8S–ITS2 region of the *Giardia* ribosomal DNA. Assemblages B (29 isolates) and C (two isolates) were identified. These results showed that *Giardia* infection can be common in chinchillas, thus spurring further molecular epizootiological studies of the infection to assess the zoonotic potential or host specificity of their isolates, to determine the source of infections, to identify the routes of transmission, and to control the infection among animal populations.

1. Introduction

The protozoan *Giardia* spp. (Family *Hexamitidae*) is an enteric flagellate which is globally widespread in a range of vertebrate hosts, including human beings, pets, livestock and wildlife (Thompson and Monis, 2004). The human infection presents with a range of clinical pictures, from asymptomatic to chronic disease (Gardner and Hill, 2001), while clinical symptoms in animal hosts may be absent to severe, and are often characterized by diarrhea and failure to thrive, particularly in young subjects (Traub et al., 2005).

The genus *Giardia* currently includes six different species, recognized on the basis of the morphology and ultrastructure of the trophozoites: *Giardia duodenalis* (syn. *Giardia lamblia*, *Giardia intestinalis*) infecting a wide spectrum of mammals as humans, pets, livestock and wildlife, *Giardia microti* and *Giardia muris* infecting rodents, *Giardia psittaci* and *Giardia ardeae* which infect birds, and *Giardia agilis*, affecting different species of amphibians (Adam, 2001). Molecular studies based on different genetic markers have demonstrated that *G. duodenalis* is a "species complex" encompassing at least eight distinct genetic groups (from A to H), the so-called assemblages (Lasek-Nesselquist et al., 2010). Therefore, it is believed that assemblages A and B have the potential for zoonotic transmission and studies on prevalence and genotypes in pets and livestock have been carried out to determine whether *G. duodenalis* can infect humans through a zoonotic route and to identify the major animal species acting as source of infection (Traub et al., 2004; Trout et al., 2004; Inpankaew et al., 2007).

The genetic identity of *Giardia* assemblages has been thoroughly investigated in several domestic animals, despite few studies have addressed the importance of the infection in non-conventional animals as, for instance, rodents, that have recently increased in popularity as pets in developed countries. Indeed, *Giardia* spp. can be commonly found in stools of wild rural rodents (Bajer, 2008; Ziegler et al., 2009) and in domestic rodents, e.g. cavies (*Cavia aperea aperea*) and hamsters (*Mesocricetus auratus*) as well (Lv et al., 2009; Gressler et al., 2010). Moreover, some evidences have shown that rodents might may harbor the zoonotic *G. duodenalis* assemblages A and B (Fayer et al., 2006; Lasek-Nesselquist et al., 2010; Lebbad et al., 2010; Beck et al., 2011).

Among captive rodents, chinchillas (*Chinchilla lanigera*), native to Chile, are becoming popular companion animals in several countries, including Italy. Although *Giardia* cysts are commonly found from veterinarians in stool of chinchillas, little is known on the epidemiology of infection, on prevalence and related risk factors and even less on the genetic identity of the isolates present in this species (Neves, 1989; Gurgel et al., 2005; Fialho et al., 2008). More specifically, only three studies have revealed infection with

* Corresponding author. Address: Department of Biopathological Sciences and Hygiene of Animal and Food Productions, Section of Parasitology, Faculty of Veterinary Medicine, University of Perugia, San Costanzo 4 Street, Postal Box 06126, Italy. Tel.: +39 075 5857740; fax: +39 075 5857743.
E-mail address: verolina2@tiscali.it (F. Veronesi).

0034-5288/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2011.12.019

assemblages A and B, thus suggesting a potential role of chinchillas as source of infectious cysts to other animals and to humans as well (Karanis and Ey, 1998; Levecke et al., 2011; Soares et al., 2011).

Given the increasing number of captive chinchillas kept as companion animals in Italy and the lack of information on the presence of giardiosis in these animals nor on the genetic identity of the isolates, the present work aimed to investigate the occurrence of the infection in animals reared to be sold as pets in Italian commercial breeding facilities and conduct a preliminary genotyping characterization of the isolates recovered.

2. Materials and methods

2.1. Animals, sampling and sample processing

From April to December 2010 three different commercial breeding facilities of chinchillas located in Central-Southern Italy were investigated on the basis of previous recovered episodes of giardiosis diagnosed by the direct observation of cysts and trophozoites in stool samples (data not shown).

Overall 104 fecal pools were collected from cages harboring a number of chinchillas ranging from 1 to 4, of different age and genders, i.e. 54 pools were collected in the facility 1, 28 in the facility 2 and 22 in the facility 3.

Each faecal sample was suspended in 10 ml of sterile phosphate – buffered saline (PBS, pH 7.2), filtered using a stainless steel mesh sieve (pore size 45 μm) and centrifuged at 1500*g* for 10 min. The supernatant was discarded and the sediment was re-suspended in an equivalent volume of 2.5% aqueous potassium dichromate ($K_2Cr_2O_7$) and stored at +4 °C until the processing in Direct Fluorescent Assay (DFA) pending further analysis.

2.2. Direct Fluorescent Antibody assay

Each stool sample (about 0.5 g) was submitted to a sucrose gradient purification (Lebbad et al., 2008) and examined by a commercial DFA (MERIFLUOR® *Cryptosporidium*/*Giardia*, Meridian) according to the manufacturer's instructions. Infection intensity was classified by shedding indices based on the average number of cysts detected per microscopic field, counting 100 fields at magnitudo 400×: L_0 for negative; L_1 ranging from 1 to 2 cysts (low excretion level); L_2 ranging from 2 to 5 cysts (moderate excretion level); $L_3 > 8$ cysts (high excretion level) as described by Xiao and Herd (1994).

2.3. Molecular procedures

All samples positive at the DFA were submitted to a PCR-coupled sequencing protocol to genotype the *Giardia* isolates present. All samples were undertaken to the DNA extraction using the QIAmp® Stool Mini Kit (QIAgen), according to the manufacturer's instructions. PCR amplification of a 315 bp fragment encompassing the ITS1–5.8S–ITS2 region in the ribosomal DNA of *Giardia* was performed according to a nested-PCR protocol described by Cacciò et al. (2010). The amplification products were separated by electrophoresis on ethidium bromide (0.1 mg/ml) stained 1.2% agarose gel and photographed with a digital camera.

PCR products were purified using the QIAquick® PCR Purification Kit (QIAgen), according to the manufacturer's instructions and sequenced in both directions on an ABI 3130 automated sequencer (Applied BioSystems). Chromatograms were examined and the sequences obtained were assembled using the software program Vector NTI Advance® (version 11.5, Invitrogen). The sequences were compared with those of the ITSs of other isolates available in the GenBank™ using the Nucleotide–Nucleotide "Basic Local Alignment Search Tool" (BLAST). Then, the ITS2 sequences were further compared with one another and the software "Molecular Evolutionary Genetics Analysis" (MEGA) 4.0 was used to calculate the nucleotide pairwise distance with the Kimura 2-parameter model among the isolates herein obtained and the corresponding ribosomal region of other 17 *Giardia* isolates available in the GenBank™ (Accession Nos. GU126449.1, GU126448.1, GU126446.1, GU126447.1, GU126443.1, GU126437.1, GU126436.1, GU126438.1, GU126440.1, GU126439.1, GU126431.1, GU126432.1, GU126433.1, GU126434.1, GU126444.1, GU126445.1, GU126435.1).

3. Results

Giardia cysts were detected by DFA in 41 out of the 104 faecal pools (39.42%, 95% CI: 30–49.5%) examined. According with the shedding grading, 19 samples (46.34%, 95% CI: 30.7–62.6%) showed a moderate number of cysts (L_2), 17 (41.46%, 95% CI: 26.3–57.9%) an high number (L_3) and 5 (12.19%, 95% CI: 41–26.2%) a low number (L_1).

A successful PCR amplification was obtained for 31 of the 41 DFA positive samples; in particular, all samples categorized as L_3 and 14 out of the 19 showing a moderate shedding grading were amplified by PCR whereas samples with low number of cysts scored negative upon PCR.

The sequence of the *Giardia* isolates herein generated showed consistency with the corresponding ribosomal region of *Giardia* available in the GenBank. Sequences were aligned over 424 nucleotidic residues and comparisons conducted by MEGA 4 software included 264 conserved sites and 97 variable sites, of which 22 were singletons and 69 were parsimony informative.

When the ITS1–5.8S–ITS2 sequences herein generated were compared with those of *Giardia* rDNA available in the GenBank™ they showed an homology ranging from 97.3% to 99% with reference animal-derived assemblage B sequences (GenBank Accession Nos. GU126439.1, GU126438.1) in 29 isolates, whereas two sequences exhibited a completely homology with a reference isolate belonging to assemblage C (GenBank Accession No. GU126443.1).

4. Discussion

There is a worldwide increasing trend to keep non exotic animals as pets and, in contrast to the information available for domestic species, few studies have addressed the importance of *Giardia* infection in these animals (Traub et al., 2004; Inpankaew et al., 2007; Vasilopulos et al., 2007). To the best of authors' knowledge this represents the first survey conducted in Italy on the occurrence of *Giardia* infection in chinchillas kept as companion animals.

The results obtained suggest that *Giardia* infection is present (i.e. infection rate of 39.42%) in animals raised in Italian commercial breeding facilities and that it frequently occurs also in presence of high shedding cyst levels. The infection rate herein found fit with those detected in previous studies conducted in Brazil and Chile (Gurgel et al., 2005; Fialho et al., 2008) but was lower respect to the 66.3% recently observed by Levecke et al. (2011) in Belgium breeding facilities despite the similar management conditions in whom the animals were maintained and the similar consistence of the studied population. The difference in the infection rate detected probably might be due to the different performances exhibited by the copromicroscopic screening by DFA used in the present study and the traditional gradient centrifugation flotation technique used in the survey carried out in Belgium (Levecke et al., 2011). Up to date there is not a standard validated diagnostic method for recovering *Giardia* infection in rodents as chinchillas,